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There is evidence suggesting that life-style factors play a significant role in breast cancer (BC) development. Insulin, which secretion is influenced by life-style factors, might represent the possible etiological linkage between lifestyle characteristics and BC. Insulin might act in the carcinogenetic process through mitogenic effect on breast epithelium. Furthermore, insulin modulates an additional proliferative and hormone-regulating factor which may be involved in the etiology of BC: the Insulin-like Growth Factor I (IGF-I).

The investigators have proposed to test the hypothesis of the linkage between serum insulin and BC in an Italian prospective cohort study conducted to investigate the role of hormones and diet in the etiology of BC (the ORDET study). 10,788 healthy volunteer women, aged 35-69, residents in Varese province, an area covered by the Lombardy Cancer Registry, were enrolled between June 1987 and June 1992. At the recruitment, blood samples were collected between 8:00 and 9:30 A.M., after an overnight fast, and stored at -80° C. During the first seven years of follow-up, the cancer registry identified 144 breast cancer cases. The study compares pre-diagnostic serum levels of insulin, IGF-I, free IGF-I, and IGF-I binding proteins (IGFBP-I and IGFBP-3) for the BC cases and 576 controls (four per BC case) randomly selected from the cohort women who did not develop BC during same follow-up period, matched on age, menopausal status, recruitment center, and recruitment period.

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## Table of Contents

## Introduction

The primary purpose of this study is to examine insulin and insulin-growth-factor pattern in relation to breast cancer etiology. In addition, it also evaluates the possible concomitant role of sex steroids in the causal relation with breast cancer.

In this prospective cohort study, 10,788 healthy volunteer women, aged 35-69, residents in Varese province, an area covered by the Lombardy Cancer Registry, were enrolled between June 1987 and June 1992. At the recruitment, blood samples were collected between 8:00 and 9:30 A.M., after an overnight fast, and stored at -80° C. During the first seven years of follow-up, the cancer registry identified 144 breast cancer cases. The proposed study will compare pre-diagnostic serum levels of insulin, IGF-I, free IGF-I, and IGF-I binding proteins (IGFBP-I and IGFBP-3) for the BC cases and 576 controls (four per BC case) randomly selected from the cohort women who did not develop BC during same follow-up period, matched on age, menopausal status, recruitment center, freezer and position within the freezer, recruitment period, and recruitment within the same daylight saving period. The proposed study represents the first attempt to evaluate the association of insulin and IGF-I with BC using data from a cohort prospective study in which several potential sources of hormone variability have been controlled for by study design.

Insulin may play an important role in the etiology of breast cancer. Insulin, in fact, is a powerful mitogenic agent inducing a dose-dependent growth response in BC cell lines acting via its own receptor. In addition, insulin may play a role in tumor promotion by upregulation of ovarian steroid secretion. In fact, chronic hyperinsulinemia has been implicated in the etiopathogenesis of hyperandrogenic status and hypothesized as a determinant of this hormonal pattern. Furthermore, insulin may act as a tumor promoter through its effect on insulin-like growth factor-I (IGF-I): IGF-I is a structural homologue of insulin, characterized by both mitogenic, and gonadotropic action of its own.

In spite of the very strong physiological evidence for a role of insulin in BC etiology, very limited evidence has been presented in epidemiological studies. To date, only two case-control studies have been conducted on serum insulin and BC risk and the results of both studies supported the association. IGF-I has also been associated with breast cancer in several case-control studies. However, these case-control studies were relatively small, blood was collected in a non-fasting state, and there was no control of other potential sources of hormone variability (i.e., circadian rhythm). The only cohort study on this question showed a seven fold elevation in BC risk between the highest and the lowest tertile of IGF-I among premenopausal women. However, this study evaluated only total IGF-I serum levels, lacking description of other potential hormone/metabolic determinants of the disease.

## Body Of Report

During the first budget year, study protocols were developed and discussed with the two institutions involved in the study: the Department of Social and Preventive Medicine at the University of Buffalo, State University of New York and the National Cancer Institute in Italy (Istituto per lo studio e la cura dei tumori). Major issues were concerned the identification and selection of all breast cancer cases and the related controls, the selection of the samples within the biological specimen bank of the cohort study, and the preparation of the computer database. The retrieval of the samples from storage and their allocation to the appropriate batches and the related sample verification was completed during this time period, so that each breast cancer case and the four related controls were handled in the same way and placed in the same shipment box. In each box, characterized by nine rows and nine columns, each case and the four related controls were placed in the same row. The sample organization allowed the laboratory for the analytical determinations, to identify the five sample to be assayed together in the same run being blind to the case-control status. This procedure has been applied to all 720 samples of the study. During the same period, detailed procedures and quality control for shipment of the samples have been discussed and organized with the shipment agency. In addition, we simulated the shipment conditions to evaluate potential changes in temperature, as a dangerous factor for the stability of the biomarkers under consideration, and the risk of thawing. We evaluated that the addition of 30 Kg. of dry ice in the shipment box would have been the safer solution. In fact, we observed that this amount of dry ice protected against temperature changes within the box ( the temperature was kept always at -87°C) for more than 16 hours from the beginning of the test. Following the verified procedure, data and samples were prepared and finally the samples were shipped from Italy to the United States. They arrived in excellent status, completely frozen. Subsequently, the samples have been stored at –80 C in mechanical freezers.

At the present time (June 2, 2000), out of the 720 samples (144 samples from breast cancer cases and 576 from control women), 320 have been already assayed for Insulin, Insulin-Like-Growth-Factor 1, Free Insulin-Like-Growth-Factor, Insulin-Like-Growth-Factor Binding Globulin 1, Insulin-Like-Growth-Factor Binding Globulin 2, Insulin-Like-Growth-Factor Binding Globulin 3. The analytical determinations for all the biomarkers will be completed in the next two months.

Publications

At the present time, there are no results or publications coming directly from this grant because we have just begun data collection. However, Dr. Muti has in press research on issue related to this grant using a previously collected data set 1,2). In the past year, she presented results regarding markers of insulin resistance and breast cancer risk and data on estrogen metabolism and breast cancer risk at the Annual Meeting of the American Association for Cancer Research (3) and at the Annual Meeting of the Society for Epidemiologic Research (4), respectively. We found that waist-to-hip ratio, the strongest index of insulin resistance in adults, was significantly associated with breast cancer risk only in premenopausal women. In addition, we found that the ratio between 2-hydroxyestrone to 16$\alpha$-hydroxyestrone was related with breast cancer protection, indicating that the metabolic shift of the estrogen metabolism toward biologically less powerful estrogens leads to a potential prevention of breast cancer. In the second study, the effect was observed again only in premenopausal women.

We have also in publication a study regarding indices of insulin resistance and risk of mortality for colon cancer. The study was conducted using an Italian dataset and we found that markers defining syndrome X (an index of insulin resistance) were associated with increase mortality for colon cancer in both men and women (5).

## Key Research Accomplishments

- Preparation of the study protocols
- Identification and selection of all breast cancer cases and related controls
- Simulation of shipment condition and verification of the temperature within the shipment box during potential 24 hrs trip
- Preparation of the sample to assure correct analytical determinations for breast cancer cases and controls and the blind fashion of the laboratory analyses
- Shipment of the samples
- Samples arrival and their storage in -80° mechanical freezers
- Biochemical analyses start
- Biochemical determination performed for half of the samples

## Reportable Outcomes

We have just begun data collection for this grant, therefore there are no reportable outcomes at this time. Analytical determinations are underway.

## Conclusions

We have just begun data collection for this grant, therefore there are no conclusions to report at this time. Analytical determinations are underway.

## References


1. Muti P., Stanulla M., Micheli A., Krogh V., Freudenheim J.L., Yang J., Schünemann H.J., Trevisan M., Berrino F. Markers of Insulin resistance and sex steroid activity in relation to breast cancer: a prospective analysis of abdominal adiposity, sebum production and hirsutism (in press, Cancer Causes Control)

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## Appendices

Paola Muti, M.D., Martin Stanulla, Andrea Micheli, Ph.D., Vittorio Krogh, Jo L. Freudenheim, Jun Yan, Holger J. Schünemann, Maurizio Trevisan, Franco Berrino

**Markers of Insulin Resistance and Sex Steroid Hormone Activity in Relation to Breast Cancer Risk: A Prospective Analysis of Abdominal Adiposity, Sebum Production, and Hirsutism**

(Cancer Causes and Control, in press)

Insulin resistance and increased levels of serum steroids have been hypothesized to be relevant etiological factors for breast cancer. Measurements of markers of insulin resistance and elevated serum steroids may identify women at high risk for breast cancer. The present study analyzed the association of breast cancer with markers of insulin resistance and elevated serum sex steroids, abdominal adiposity, increase in sebum production and hirsutism in a case-control study nested in a prospective cohort study.

Between 1987 and 1992, 10,786 women (aged 35-69) were recruited in a prospective study on breast cancer in Italy, the ORDET study. Women with history of cancer and on hormone therapy were excluded at baseline. At recruitment, abdominal adiposity was calculated from the ratio of waist-to-hip circumferences. Sebum production was measured on the forehead under standardized conditions using a sebumeter. Nine androgen-sensitive body areas were evaluated for hirsutism and a total hirsutism score was computed. After an average of 5.5 years of follow-up, 144 breast cancer cases were identified among the participants of the cohort. For each breast cancer case, four matched controls were randomly chosen from members of the cohort who did not develop breast cancer during the follow-up period.

Waist-to-hip ratio was associated with breast cancer in premenopausal women: age and body mass index (BMI) adjusted relative risk (RR) for the highest tertile of waist-to-hip ratio was 2.2 [95% Confidence Interval (CI) 1.04 – 4.75], p for trend 0.03. In the analysis conducted within strata of BMI, the effect of waist-to-hip ratio was confined to the group of thinner women: RR for the highest tertile of waist-to-hip ratio was 3.4 (95% CI 1.2 – 9.5).

Sebum production and hirsutism were associated with breast cancer among postmenopausal women. Age and BMI adjusted RRs for the upper tertiles were 2.2 (95%CI 1.1 – 4.6), p for trend 0.01, and 2.3 (95% CI 1.1 – 4.9), p for trend 0.03, for sebum and hirsutism, respectively.

These results add evidence for a role of hormones and metabolic alterations in breast cancer etiology and for different relations of these risk factors with breast cancer in premenopausal and postmenopausal women.

Paola Muti , H. Leon Bradlow , Andrea Micheli , Vittorio Krogh, Jo L. Freudenheim, Holger J. Schünemann, Martin Stanulla, Jun Yang, Daniel W. Sepkovic, Maurizio Trevisan , Franco Berrino

**Estrogen Metabolism and Risk of Breast Cancer: A Prospective Study of the 2:16α-Hydroxyestrone Ratio in Pre- and Postmenopausal Women**
(Epidemiology, in press)

Experimental and clinical evidence suggests that 16α-hydroxylated estrogen metabolites, biologically strong estrogens, are associated with breast cancer risk, while 2-hydroxylated metabolites, with lower estrogenic activity, are weakly related to this disease. This study analyzes the association of breast cancer risk with estrogen metabolism, expressed as the ratio of 2-hydroxyestrone to 16α-hydroxyestrone, in a prospective nested case-control study. Between 1987 and 1992, 10,786 women (aged 35-69) were recruited to a prospective study on breast cancer in Italy, the "hORmones and Diet in the ETiology of breast cancer" (ORDET) study. Women with a history of cancer and women on hormone therapy were excluded at baseline. At recruitment, overnight urine was collected from all participants and stored at -80°C. After an average of 5.5 years of follow-up, 144 breast cancer cases and four matched controls for each case were identified among the participants of the cohort. Among premenopausal women, a higher ratio of 2-hydroxyestrone to 16α-hydroxyestrone at baseline was associated with a reduced risk of breast cancer: women in the highest quintile of the ratio had an adjusted odds ratio (OR) for breast cancer of 0.58 [95% confidence interval (CI) 0.25–1.34]. The corresponding adjusted OR in postmenopausal women was 1.29 (95% C.I. 0.53-3.10). Results of this prospective study support the hypothesis that the estrogen metabolism pathway favoring 2-hydroxylation over 16α-hydroxylation is associated with a reduced risk of invasive breast cancer risk in premenopausal women.